Smoking is associated with impaired long-term glucose metabolism in patients with type 1 diabetes mellitus

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Abstract: BACKGROUND AND AIMS: Smoking is known to negatively influence glucose metabolism both in healthy subjects and in patients with diabetes. The aim of this study was to compare glycemic control in patients with type 1 diabetes mellitus who were smokers with those who did not smoke during a prospective long-term follow-up. METHODS AND RESULTS: In a single center, 763 patients with type 1 diabetes mellitus were included, 160 (21.0%) of them were smokers. Patients were treated with intensive insulin therapy according to existing guidelines. Glucose control was monitored quarterly, diabetes related complications and cardiovascular risk factors were assessed at least once a year. Glucose control in smokers was significantly worse than in non-smokers at baseline and during follow-up (mean HbA1c during 5047 patient-years of follow-up 7.9 ± 1.3% in smokers and 7.3 ± 1.1% in non-smokers, $p < 0.001$) despite a higher insulin dosage in smokers (0.71 ± 0.30 U/kg vs. 0.65 ± 0.31 U/kg in non-smokers, $p = 0.046$). HDL cholesterol was lower in smokers at baseline (1.53 ± 0.45 vs. 1.68 ± 0.51 in non-smokers, $p = 0.048$). Diabetes related complications tended to occur with a higher frequency in smokers, with a significant difference in macroalbuminuria (9.8% vs. 4.8% in non-smokers, $p = 0.047$). CONCLUSION: Smoking is associated with worse glucose control in patients with type 1 diabetes mellitus despite the same treatment strategies as in non-smokers. Hyperglycemia, therefore, may contribute to an earlier incidence of diabetes related complications in these patients, in addition to direct toxic effects of smoking.

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Smoking is associated with impaired long-term glucose metabolism in patients with type 1 diabetes mellitus

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Methods and Results: In a single center, 763 patients with type 1 diabetes mellitus were included, 160 (21.0%) of them were smokers. Patients were treated with intensive insulin therapy according to existing guidelines. Glucose control was monitored quarterly, diabetes related complications and cardiovascular risk factors were assessed at least once a year. Glucose control in smokers was significantly worse than in non-smokers at baseline and during follow-up (mean HbA1c during 5047 patient-years of follow-up 7.9 ± 1.3% in smokers and 7.3 ± 1.1% in non-smokers, $p < 0.001$) despite a higher insulin dosage in smokers (0.71 ± 0.30 U/kg vs. 0.65 ± 0.31 U/kg in non-smokers, $p=0.046$). HDL cholesterol was lower in smokers at baseline (1.53 ± 0.45 vs. 1.68 ± 0.51 in non-smokers, $p=0.048$). Diabetes related complications tended to occur with a higher frequency in smokers, with a significant difference in macroalbuminuria (9.8% vs. 4.8% in non-smokers, $p=0.047$).

Conclusion: Smoking is associated with worse glucose control in patients with type 1 diabetes mellitus despite the same treatment strategies as in non-smokers. Hyperglycemia, therefore, may contribute to an earlier incidence of diabetes related complications in these patients, in addition to direct toxic effects of smoking.

Introduction

Diabetes mellitus and tobacco smoking are both major cardiovascular risk factors (1, 2). Furthermore, smoking and diabetes do not compromise cardiovascular health independently. There is a well known association of smoking with insulin resistance (3-6) and an increased risk for the development of type 2 diabetes mellitus (7, 8) in patients at risk - in particular those exhibiting other features of the metabolic syndrome.

In patients with an already established diagnosis of type 2 diabetes mellitus, smoking seems to worsen insulin resistance and glycemic control (9, 10). There are also cross-sectional multi-center studies suggesting an association of smoking with impaired metabolic control in patients with type 1 diabetes mellitus (9, 11-13). However, prospective longitudinal data linking glycemic control with smoking in patients with type 1 diabetes mellitus is missing. It is, therefore, not known if impaired metabolic control is due to direct effects of smoking or if this finding is the result of associated differences (e.g. attitude towards diabetes therapy, frequency of consultation, adherence to therapy) between smokers and non-smokers.

In order to address this important issue, we conducted a prospective single-center study in a cohort of patients with type 1 diabetes mellitus, consisting of non-smokers and current smokers. We hypothesized that possible differences in metabolic control may persist even after initiation of intensive diabetes therapy when smoking was not ceased.

Methods

Study design and population. We conducted a prospective cohort study by continuously including all patients with known type 1 diabetes mellitus who were referred to the outpatient clinic of the department of Endocrinology, Diabetes and Clinical Nutrition at the University Hospital of Zurich, a tertiary referral center, and were followed there for at least one year. Written informed consent was obtained from every patient included in the study. The study was conducted over 16 years from January 1994 until December 2009.

Patients were interrogated about smoking habits at the first visit and then annually (non-smokers) or at every consultation (smokers). Because the study was not designed as a cross-over study, patients changing their smoking habits during the study (e.g. start or stop of smoking) were no longer included in the study.

Primary endpoint of the study was metabolic control, assessed by glycosylated hemoglobin (HbA1c) measurements. Secondary endpoint was the presence of diabetes related complications.

Patient management and follow-up. Patients were followed by an interdisciplinary team consisting of diabetologists, diabetes educators and nutritionists. Treatment was conducted according to the description of the methodology of the Diabetes Control and Complications Trial (DCCT) (14) either by multiple daily injections of insulin (at least 4 to 5 injections) or continuous subcutaneous insulin infusion system (CSII). The goal of therapy was to achieve an HbA1c of 7% or less without the occurrence of frequent episodes of severe hypoglycemia. Patients were seen at least once every 3 to 4 months.

All current smokers were advised to stop smoking and were offered help in doing so (e.g. nicotine replacement therapies, tobacco dehabituation program). Changes in smoking habits were registered as mentioned above.

Blood glucose control as assessed by HbA1c and daily blood glucose self-measurements performed by the patients was analyzed at every visit. We also assessed hypoglycemic events as well as blood

pressure, heart rate and body weight at every visit. Retinopathy, neuropathy or albuminuria as well as serum lipids and kidney function were assessed once yearly or more frequently if necessary.

Cardiovascular risk factors, i.e. dyslipidemia and blood pressure, were treated according to the existing ADA (American Diabetes Association) guidelines. ACE (angiotensin converting enzyme) inhibitor- or AII (angiotensin II) antagonist-therapy was introduced if microalbuminuria was confirmed or if blood pressure was above current recommended target values.

The socio-economic situation of the patients, e.g. marital status, profession and education (classified according to the International Standard Classification of Education ISCED) was recorded at study entry and updated during the study if necessary.

Biochemical analysis. HbA1c was measured with the DCA 2000 (Bayer Diagnostics, Elkhart, USA) according to the manufacturer's instructions. Measurement of microalbuminuria was done by an overnight urine sample using the Micral Test II (Boehringer-Mannheim) until 1998 (6.4% of measurements in this study) and afterwards in a spot urine with the DCA 2000 (93.6% of measurements).

Cholesterol was measured by an enzymatic colorimetric test using cholesterol esterase and cholesterol oxidase, triglycerides were determined by a colorimetric reaction with iodonitrotetrazolium chloride after enzymatic hydrolysis (modular P lab analyzer, Roche, Switzerland). HDL was measured by a homogeneous enzymatic test (Cobas Integra lab analyzer, Roche, Switzerland). LDL was calculated with the Friedewald formula (15).

Clinical outcome measures. Body weight was measured to the nearest kilogram, height to the nearest centimeter. BMI (body mass index) was calculated as weight/height^2 (kg/m^2). Blood pressure was recorded after 5 min in the sitting position during interview with a mercury sphingomanometer.

Severe hypoglycemia was defined as need of assistance by another person to correct the hypoglycemia, including coma and seizures.

Microalbuminuria was defined as presence of urinary albumin excretion > 20 µg/min, corresponding to 30 mg/24 hours, or an urinary albumin/creatinine ratio > 3.5 mg/mmol for females (2.5mg/mmol

for males), macroalbuminuria was defined as presence of urinary albumin excretion > 200 µg/min, corresponding to 300 mg/24 hours, or an urinary albumin/creatinine ratio > 35 mg/mmol for females (25mg/mmol for males) (16).

Neuropathy was evaluated using the Michigan Neuropathy Screening Instrument (MNSI) (17). Patients were diagnosed with neuropathy if the MNSI score exceeded 2.

Diabetic retinopathy (proliferative or non-proliferative) with or without need for intervention (laser therapy, vitrectomy) was diagnosed by retinal examination by an ophthalmologist.

The presence of macrovascular complications was evaluated by taking the medical history about past myocardial infarction or cardiac intervention (angioplasty or bypass surgery), cerebral ischemia (transient ischemic attack, stroke) or periphereal vascular disease with need for intervention (angioplasty, amputation).

Termination of follow-up. Reasons for termination of follow-up in this study were change of smoking habits (e.g. start or stop of smoking) and discontinuation of the therapy at our institution (change of the treating institution, place of residence or death of the patient).

Statistical analysis. Data are described as mean ± standard deviation, median (1^{st}, 3^{rd} quartile) or relative frequency. For the analysis of independent categorical frequency data, the χ^2 test was applied, and for related categorical frequency data, a McNemar test was performed. For comparison of continuous variables in two independent groups, the Mann–Whitney test was used, for related samples, the Wilcoxon test was applied. A generalized linear model was used to test the influence of multiple factors on target values. The Bonferroni correction was applied to address the problem of multiple comparisons. A value of $p<0.05$ was considered significant. The statistical analyses were performed using SPSS 18.0 software (SPSS Inc, Chicago, USA) for Windows (Microsoft, Redmond, USA).

Results

Patient characteristics and follow-up. 763 patients were included in the study, follow-up was 5.8 (2.7, 9.6) years and 5.5 (2.3,10.0) years in non-smokers and smokers, respectively ($p = 0.46$). The patient numbers during follow-up are given in figure 1. 252 patients were seen for ≥ 10 years. Total patient-years of the follow-up was 5047.

Patient characteristics of the whole collective as well as of smokers and non-smokers are shown in table 1. Patients included in the cohort had a mean age of 35.9 ± 13.9 years, diabetes existed for a mean of 13.0 ± 12.2 years. Mean BMI at study entry was 23.8 ± 5.6 kg/m^2.

At study entry, 160 patients were smokers (21.0%). There was no significant difference in any of the characteristics mentioned between smokers and non-smokers. However, gender distribution between the two groups differed significantly (52.1% male in non-smokers, 71.3% male in smokers, $p < 0.001$).

Smoking habits. Means of smoking was cigarette smoking in 96.8% of smokers, with 1.3% smoking pipe and 1.9% cigars.

Cigarette smokers had a smoking history of 21.6 ± 14.8 pack-years at the beginning of follow-up. 24 patients (15.0%) quitted smoking during the study, 6 non-smokers started smoking (1.0%).

Metabolic control. The yearly mean HbA1c values during follow-up are depicted in figure 1. HbA1c levels could be lowered significantly in both groups when baseline values were compared with results one year after study entry (from 8.1 ± 2.0% to 7.3 ± 1.2% in non-smokers and from 9.0 ± 2.3% to 7.9 ± 1.5% in smokers, $p < 0.001$ in both groups) and remained stable thereafter, but differed significantly at baseline ($p<0.001$) and during the entire follow-up (mean HbA1c during follow up was 7.3 ± 1.1% in non-smokers and 7.9 ± 1.3% in smokers, $p < 0.001$). The proportion of patients who achieved a target of 7% or less (mean during follow-up) was 42.3% in non-smokers and 23.8% in smokers ($p < 0.001$). Due to the gender difference of the smoker- and non-smoker group, we separated the influence of gender and smoking habit in a generalized linear model. For the baseline measurement as

well as for every year of follow-up, HbA1c differed between smokers and non-smokers. When the Bonferroni correction for multiple comparisons was applied in this model, HbA1c levels remained significantly different until year 5 of follow-up.

The 24 patients who quitted smoking during the study were excluded from prospective data analysis. However, changes of HbA1c levels in these patients were analyzed post-hoc. The yearly mean HbA1c changed by -0.5 ± 0.9% from before to after smoking cessation (p=0.055).

At baseline, HbA1c was equal in males and females (8.3 ± 2.1% for both, p = 0.44 when adjusted for smoking), but females showed significantly worse control during follow-up (mean HbA1c during follow up 7.5 ± 1.3% in females and 7.4 ± 1.1% in males, p = 0.008 when adjusted for smoking) until year 5 of follow-up.

Diabetes associated complications and cardiovascular risk factors. The presence of diabetes related complications before and after follow-up is shown in table 2. There was a tendency towards a higher frequency of every single micro- and macrovascular complication in smokers as compared to non-smokers. A significant difference between the two groups was seen with regard to macroalbuminuria at the end of follow-up (4.8% in non-smokers and 9.8% in smokers, p=0.047). When adjusted for mean HbA1c during follow-up, this difference was no longer persistent. The frequency of every complication increased in the whole cohort during follow-up.

Weight, blood pressure and serum lipids were not different between smokers and non-smokers before and after follow-up with the exception of HDL cholesterol at the start of follow-up (1.68 ± 0.51 mmol/l in non-smokers, 1.53 ± 0.45 mmol/l in smokers, p=0.048) (table 3). This difference was still persistent at the end of follow-up but was no longer significant at this point (p=0.07). There was a significant weight gain in both groups during follow-up. Systolic blood pressure decreased in non-smokers and diastolic blood pressure decreased in both groups. Serum lipid levels did not change during follow-up with the exception of total cholesterol, which was lowered in smokers.

Insulin therapy and treatment of cardiovascular risk factors. 73.5% of patients were treated with multiple daily insulin injections of a short- and a long-acting insulin, 26.5% of patients were treated

with continuous subcutaneous insulin infusion (CSII; insulin pump). The rate of CSII treatment was 27.9% in non-smokers and 21.2% in smokers (p=0.15). HbA1c was not significantly influenced by the use of insulin pump therapy (mean during follow-up 7.5 ± 1.3% without insulin pump, 7.3 ± 1.0% with insulin pump, p=0.32).

Insulin dosage was comparable between the two groups at the start of follow-up (0.61 ± 0.28 U/kg in non-smokers vs. 0.58 ± 0.27 U/kg in smokers, p=0.65), but during follow-up the extent of increase in insulin dosage in the smoker group was significantly larger as compared to non-smokers (+0.13 ± 0.28 U/kg vs. +0.05 ± 0.30 U/kg, p=0.002). At the end of follow-up, insulin dosage was significantly higher in smokers (0.71 ± 0.30 U/kg vs. 0.65 ± 0.31 U/kg in non-smokers, p=0.046). 21.6% of non-smokers and 26.3% of smokers were treated with a statin at the end of follow-up (p=0.21), and 33.2% of non-smokers and 36.9% of smokers with an ACE inhibitor or AII antagonist due to hypertension or albuminuria (p=0.38).

Occurrence of severe hypoglycemia. There was no difference in the number of patients experiencing one or more severe hypoglycemias (grade II or III) between groups during follow-up (27.4% of non-smokers, 28.8% of smokers, p=0.33), the absolute number of hypoglycemic episodes, in which assistance was required, was 16 per 100 patient-years in the non-smoker group and 17 per 100 patient-years in the smoker group.

Relation of socio-economic status and smoking. The prevalence of smoking did not depend on marital status (single, married, divorced, widowed; p=0.22) or professional activity (employed, unemployed, retired, student; p=0.15). However, frequency of smoking was significantly different (p=0.02) when the educational level was compared with a percentage of smokers in ISCED level 0-3 (compulsory education / apprenticeship) of 28.2%, in level 4-5B (university of applied science) of 14.8% and in level 5A-6 (university) of 12.3%. Furthermore, educational level was the only socio-economic factor that was associated with differences in HbA1c when separated from the effect of smoking by a generalized linear model (mean HbA1c during follow-up of 7.5 ± 1.2% in ISCED level 0-3, 7.3 ± 1.2% in ISCED level 4-5B and 7.0 ±1.1% in ISCED level 5A-6). However, smoking remained

statistically significantly associated with HbA1c when separated from the effects of gender and socio-economic status (p=0.009).

Mortality. 5.0 death per 1000 patient-years occurred during follow-up (total 25) with no significant difference between non-smokers (5.2) and smokers (3.9; p=0.75).

Discussion

In this long-term cohort study (more than half of the patient number of the DCC trial with the same duration of follow-up), smoking could be identified as a major risk factor for worse metabolic control in patients with type 1 diabetes mellitus. HbA1c levels as an indicator of glucose control were significantly different between smokers and non-smokers at the time point of study entry, a difference that persisted even after years of intensive insulin therapy at the same institution.

This therapy resulted in HbA1c levels in the non-smoker group that are comparable with those of the intensive-therapy group in the DCCT (18), indicating that such results can be achieved outside a major trial design in an outpatient setting of a larger hospital. Interestingly, the number of severe hypoglycemias was about the same as reported from the conventional-therapy group of the DCCT (19 per 100 patient-years) and therefore much lower as in the intensive-therapy group of the DCCT (62 per 100 patient-years). The smoker group had a higher HbA1c already at the start of follow-up. This difference could be reduced, but not eliminated despite a considerably greater increase of the insulin dosage with a higher total insulin dose at the end of follow-up in the smoker group. Furthermore, the proportion of patients achieving a target HbA1c value of 7% or less was almost twice as high in the non-smoker group than in the smoker group. In accordance with these findings, there was a statistically non-significant tendency of a decrease in HbA1c in patients who quitted smoking. The occurrence of severe hypoglycemia did not differ between the two groups.

The observation that a higher insulin dosage leads to a less tight glucose control with the same rate of severe hypoglycemias suggests that smoking impairs glucose homeostasis by short-term changes of insulin sensitivity, an effect of smoking already described by earlier experimental studies in healthy volunteers (3), which is unpredictable most of the time. Another possible explanation of our observations could be the vasoconstriction mediated delay in insulin absorption from subcutaneous tissue by smoking. Especially the accordance of prandial insulin and postprandial blood glucose elevations may be disturbed by this effect (19).

In contrast to glycemic control, most other cardiovascular risk factors could be adequately controlled in both groups: There was no significant difference in serum total and LDL-cholesterol, triglycerides

or blood pressure between the two groups. However, HDL-cholesterol in smokers was 9 % lower at the start of follow-up. The negative correlation of smoking and HDL-cholesterol is well known (20). Impaired insulin action (21, 22) and lower HDL (23, 24) both contribute to a phenotype resembling the metabolic syndrome and are considered important factors that determine the risk of this syndrome in all age groups.

Interestingly, weight and weight change did not differ between the two groups. The observed (non-significant) tendency of more frequent use of ACE inhibitors / AII antagonists and statins in smokers is likely to be the result of the adverse effects of smoking on blood pressure, albuminuria and serum lipids.

Increased morbidity in patients with type 1 diabetes depends predominantly on the occurrence of diabetes related complications. There was a tendency of a higher prevalence of microvascular and macrovascular diabetes related complications in smokers. However, only the presence of macroalbuminuria differed significantly between the two groups. This difference did not persist when adjusting for mean HbA1c during follow-up, indicating that the difference of the two groups may at least partly be explained by the difference in glycemic control. Nevertheless, it should be emphasized that earlier studies have shown that smoking influences nephropathy (probably by direct toxic effects on the endothelial function) (11, 25-27) and neuropathy (28) also independently of glycemic control, whereas data on retinopathy is conflicting (11, 29). In addition, low HDL levels (as seen in the smoker group) are associated with a higher incidence of chronic kidney disease in patients with type 2 diabetes mellitus (30).

Mortality (5.0/1000 patient-years) during the study did not differ between the two groups and was only marginally higher than expected in a age adjusted general population in Switzerland (4.3/1000 patient-years) (31).

The prevalence of every complication increased in the whole cohort during the study despite good glycemic control, pointing to the concept of “glucose legacy” (32).

Of interest, glycemic control was slightly, but significantly worse in female patients compared to males during follow-up, a difference that was not present at the start of follow-up. This observation is consistent with earlier reports on glucose control that described a gender-dependent difference with

worse control in females occurring after the transfer of adolescents to an adult-focused diabetes program (32).

The strength of this study is its prospective single-center design and the high number of patient-years. There are also limitations. Due to the known deleterious effects of smoking, a study as presented here is not randomized, which leads to the possible bias of unequal groups. One major difference that we could determine was a difference in gender (with more males in the smoker group). However, by treating gender as an additional factor that possibly affects the investigated outcome, we separated its effect in our analysis from the effect of smoking itself, thereby minimizing a possible bias. The possible influence of other factors, e.g. of socio-economic status or lifestyle, is more difficult to assess. Our analysis revealed that educational level inversely correlates with smoking habits and HbA1c, this is in accordance with observations in many previous studies (31, 33, 34). However, when separating the effect of smoking from the effect of educational level, both factors independently influenced glycemic control.

In summary, this study demonstrates that, in contrast to other cardiovascular risk factors that are less difficult to treat (e.g. dyslipidemia, hypertension), achieving good glucose control is more difficult in patients with type 1 diabetes mellitus who smoke despite the same intensive insulin treatment strategies as in non-smokers. Hyperglycemia, therefore, may contribute to an earlier incidence of diabetes related complications in patients with type 1 diabetes who smoke, in addition to the direct toxic effects of smoking, and therefore increase morbidity in this population. This emphasizes the importance of counseling for smoking cessation in these patients, perhaps with special emphasis concerning groups with a higher prevalence of smoking (e.g. patients with lower educational level).

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1 Tables

2

3 Table 1: Patient characteristics of patients included in the study

4

Characteristic	Non-Smokers	Smokers	Total	p
n	603	160	763	
Sex, male/female (%)	52.1	71.3	56.1	< 0.001
Age (y)	36.1 ± 14.2	35.1 ± 12.8	35.9 ± 13.9	0.66
Diabetes duration (y)	13.4 ± 12.5	11.5 ± 10.8	13.0 ± 12.2	0.16
BMI (kg/m^2)	23.9 ± 5.8	23.6 ± 4.5	23.8 ± 5.6	0.45
Follow-up (y)	5.8 (2.7, 9.6)	5.5 (2.3,10.0)	5.7 (2.7, 9.7)	0.46

5

6 Data are means ± SD, median (1^{st}, 3^{rd} quartile) or frequency (%).

Table 2: Presence of diabetes related complications in patients participating in the study before and after follow-up

Complication		Non-Smokers	Smokers	Total	p †
Nephropathy (microalbuminuria) (%)	Start	18.3	21.1	18.9	0.55
	End	28.6 *	32.5 *	29.4 *	0.26
Nephropathy (macroalbuminuria) (%)	Start	3.8	7.3	4.5	0.11
	End	4.8	9.8	5.9 *	0.047
Neuropathy (%)	Start	9.7	17.7	11.3	0.09
	End	17.3 *	23.4 *	18.5 *	0.10
Retinopathy (%)	Start	24.4	28.8	25.3	0.31
	End	35.7 *	40.6 *	36.7 *	0.22
Macrovascular disease (%)	Start	2.8	4.4	3.1	0.87
	End	8.0 *	11.9 *	8.8 *	0.37

Data are frequency (%). A generalized linear model was applied to separate the influence of smoking and gender on complication prevalence. † comparison between groups. * change of prevalence at end of follow-up compared to start in the same group ($p < 0.05$).

Table 3: BMI, blood pressure and serum lipids in patients participating in the study before and after follow-up

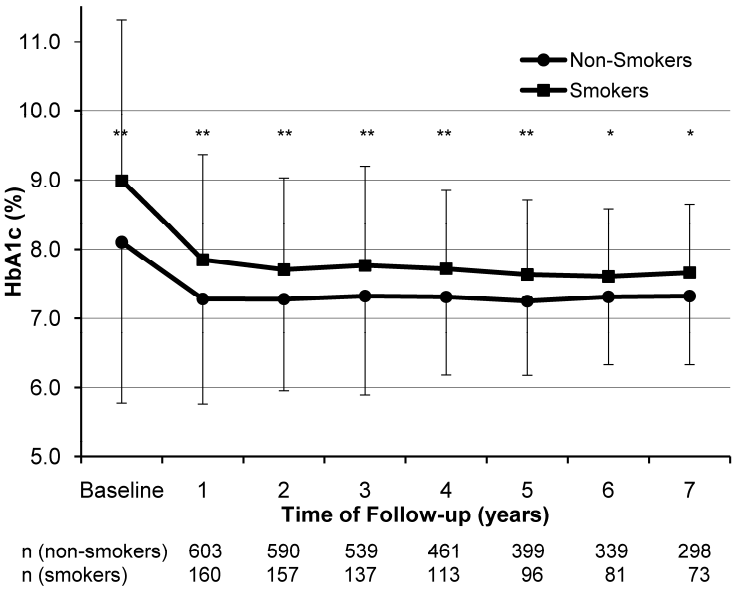
Parameter		Non-Smokers	Smokers	Total	p †
BMI (kg/m^2)	Start	23.8 ± 3.6	23.7 ± 4.5	23.8 ± 3.8	0.78
	End	25.0 ± 4.1*	25.4 ± 5.6*	25.1 ± 4.4*	0.55
Systolic blood pressure (mmHg)	Start	127.8 ± 16.7	127.7 ± 18.0	127.8 ±17.0	0.90
	End	126.2 ± 15.7*	127.3 ± 18.6	126.5 ± 16.3*	0.71
Diastolic blood pressure (mmHg)	Start	79.9 ± 10.8	79.6 ± 13.3	79.8 ± 11.4	0.64
	End	77.9 ± 10.3*	76.8 ± 12.3*	77.7 ± 10.7*	0.09
Total cholesterol (mmol/l)	Start	4.98 ± 1.07	5.03 ± 1.04	4.99 ± 1.06	0.28
	End	4.86 ± 1.04	4.80 ± 0.98*	4.85 ± 1.03*	0.83
HDL cholesterol (mmol/l)	Start	1.68 ± 0.51	1.53 ± 0.45	1.65 ± 0.50	0.048
	End	1.68 ± 0.51	1.53 ± 0.46	1.65 ± 0.50	0.07
LDL cholesterol (mmol/l)	Start	2.70 ± 0.92	2.78 ± 1.00	2.72 ± 0.93	0.60
	End	2.73 ± 0.92	2.59 ± 0.78	2.71 ± 0.90	0.15
Triglyceride (mmol/l)	Start	1.20 ± 0.87	1.33 ± 0.85	1.23 ± 0.86	0.25
	End	1.21 ± 0.84	1.27 ± 0.82	1.22 ± 0.83	0.62

Data are means ± SD. A generalized linear model was applied to separate the influence of smoking and gender. † comparison between groups. * change of parameter at end of follow-up compared to start in the same group ($p < 0.05$).

Figure 1: Legend

HbA1c levels in smokers (squares) and non-smokers (circles) before and during follow-up. ** $p<0.001$ (significantly different after Bonferroni correction), * $p<0.05$ (not significantly different after Bonferroni correction). A generalized linear model was applied to separate the influence of smoking and gender on HbA1c. n indicates patients included until this time point.

Figure 1


11.0
10.0
9.0
8.0
7.0
6.0
5.0
HbA1c (%)
Non-Smokers
Smokers
** ** ** ** ** ** * *
Baseline 1 2 3 4 5 6 7
Time of Follow-up (years)
n (non-smokers) 603 590 539 461 399 339 298
n (smokers) 160 157 137 113 96 81 73